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Master Thesis no. 254
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Cover: Experimental plot, photo by Stina Söderlund,

Abstract

The dry tropical forests are among the most threatened ecosystems in the world; they have been degraded and removed due to commercial values and cattle ranges. Restoration projects have gained global importance, including in Costa Rica where large restoration efforts have been started since 1970s. However, previous restoration projects have scored limited success in several areas due to species mismatch with the prevailing site conditions. Thus, trait-based approach has been emerged as a way to identify candidate species for restoration. A trait is a morphological, anatomical, biochemical, physiological and/or phenological feature measurable at the individual level; whereas a functional trait is any trait which impacts fitness indirectly by its effects on growth, reproduction and survival. The aims of the study were to examine variation in traits within (intra-specific) and between (inter-specific) species in relation to above- (light) and below-ground (nutrient and water) resources; and if functional traits are good predictors of survival and growth of planted seedlings of four tree species, *Astronium graveolens* (RonRon), *Dalbergia retusa* (Cocobolo), *Hymenaea courbaril* (Guapinol), and *Thouinidium decandrum* (Sardino). For this purpose, a factorial experiment involving two levels of forest type (old versus young) and trenching (with versus without) was established in the forest experimental station, Horizontes, a part of Conservation area of Guanacaste. There were four plots in each forest type, and four seedlings of each species were planted in May 2013 in each sub-plot. Leaf- and stem functional traits and survival rate were assessed two years post-planting while root collar diameter and height of seedlings were recorded for two consecutive years post-planting. Two-way analysis of variance (ANOVA) was performed to determine significant differences in intra-specific trait variabilities while three-way ANOVA was performed to determine differences in traits and seedling performance among species and treatments. To examine whether traits can predict field performance of planted seedlings, correlation analyses were performed between traits and seedling growth variables.

The results showed that intra-specific leaf and stem traits did not vary significantly between forest types and trenching treatment for all the studied species, except *A. graveolens* for which specific leaf area was significantly higher ($p = 0.015$) for seedlings planted under old- than young forest. Three-way ANOVA showed highly significant differences in leaf- and stem traits among species ($p < 0.01$), except stem dry matter content ($p = 0.209$). Leaf thickness, leaf dry matter content and specific leaf area did also significantly vary between forest types ($p < 0.05$) but not between trenching treatments and interactions between species, forest type and trenching treatments. The mean stem specific density, leaf dry matter content and leaf density were lower while specific leaf area was higher for *D. retusa* than other species. *H. courbaril* had higher leaf thickness than the rest of the species. For *A. graveolens* and *T. decandrum*, stem specific density, specific leaf area, leaf dry matter content and leaf density did not differ significantly. Among the traits analysed, leaf thickness and leaf dry matter content were significantly higher for seedlings planted in young- than old forest; whereas specific leaf area was significantly lower for seedlings planted in young- than old forest. The trenching treatment (with versus without) had no effect on inter-specific traits.

The correlation between inter-specific traits and seedling growth was significant for most of the traits analysed. Seedling height and root collar diameter during the first two years post-planting significantly and positively correlated with leaf thickness and negatively with specific leaf area. The current annual increment in height (CAIH) and root collar diameter (CAID) were positively correlated with leaf density and leaf dry matter content but negatively correlated with specific leaf area. Correlation analysis between intra-specific traits and seedling growth was also performed; and significant correlation was found between leaf thickness and stem dry matter content with seedling growth in three out of four species; especially leaf thickness was correlated with both diameter and height growth of two out of four species.

In conclusion, this study highlights the importance of functional traits when looking on survival and growth of different species in different environmental conditions. This new trait-based approach is shown to predict survival and growth and thereby can be used as a possible guide for future restoration projects in similar climatic- and environmental conditions. As sample sizes were few in the present study, I recommend further research using larger sample size for each treatment (sub-plot) and more species to be able to identify the best predictive traits.

Keywords: Functional traits, Leaf traits, Restoration, Stem traits, Tropical Dry Forests

Sammanfattning

Den tropiska torrskogen är en av de mest hotade ekosystemen i världen. De har degraderats och arealen minskar på grund av skogarnas kommersiella värden, ökad odling av jordbruksgrödor och boskapsskötsel. Restaureringsprojekt har fått global betydelse, däribland i Costa Rica där stora restaureringsinsatser inleddes på 1970-talet. Emellertid har restaureringsprojekten visat begränsad framgång inom flera områden på grund av att använda arter varit i obalans med rådande förhållanden på platsen. Sålunda har ett egenskaps-baserat tillvägagångssätt vuxit fram som ett sätt att identifiera kandidatarter för restaurering. Egenskaper av betydelse i sammanhanget kan vara morfologiska, anatomiska, biokemiska, fysiologiska och fenologiska, mätbara på individnivå. En funktionell egenskap är en egenskap som påverkar lämpligheten indirekt genom dess effekter på tillväxt, reproduktion och överlevnad. Syftet med studien var att undersöka variationer i egenskaper inom arten (intra-specifik) och mellan arter (inter-specifika) i förhållande till ovanjordiska- (ljus) och underjordiska (näringsämnen och vatten) resurser, samt om de funktionella egenskaperna är goda prediktorer för överlevnad och tillväxt av planterade plantor av fyra trädslag, *Astronium graveolens* (Ronron), *Dalbergia retusa* (Cocobolo), *Hymenaea courbaril* (Guapinol), och *Thouinidium decandrum* (Sardino). För detta ändamål lades ett faktorsexperiment ut i två skogstyper (gammal eller ung), med eller utan rotisolering (diken) i försöksskogar i Horizontes, en del av ett naturvårdsområde i Guanacaste, Costa Rica. På fyra ytor i varje skogstyp planterades fyra plantor av varje art i maj 2013. Blad- och stamfunktionella egenskaper och överlevnad mättes två år efter plantering och rothalsdiameter och höjd mättes varje år under två år efter plantering. Två-vägs variansanalys (ANOVA) användes för att beräkna skillnader i variation i inomartsspecifika egenskaper, och trevägs ANOVA användes för att beräkna skillnader i variation i egenskaper mellan arter och behandlingar. För att undersöka om egenskaper kan förutsäga överlevnad och tillväxt i fält för planterade plantor utfördes korrelationsanalyser mellan egenskaper och tillväxtvariabler.

Resultaten visar att inomartsspecifika blad- och stamegenskaper inte varierar signifikant mellan skogstyper och dikningsbehandlingar för alla studerade arter, förutom för *A. graveolens* för vilken den specifika bladytan var signifikant högre ($p = 0,015$) för plantor planterade i den äldre skogstypen än i ungskog. Trevägs ANOVA visade starka signifikanta skillnader i blad- och stamegenskaper mellan arter ($p < 0,01$), förutom stamtorrsubstanshalt ($p = 0,209$). Bladtjocklek, bladtorrsubstanshalt och specifik bladyta varierade signifikant mellan skogstyper ($p < 0,05$), men inte mellan dikningsbehandlingar och interaktioner mellan arter, skogstyp och dikningsbehandlingar. Medelvärdet för stamspecifik densitet, bladtorrsubstanshalt och bladdensitet var lägre medan specifika bladytan var högre för *D. retusa* jämfört med de andra arterna. *H. courbaril* hade högre bladtjocklek än övriga arter. Stamspecifik densitet, specifik bladyta, bladtorrsubstanshalt och bladtäthet skilde sig inte signifikant mellan *A. graveolens* och *T. decandrum*. Bland de egenskaper som analyserades, var bladtjocklek och bladtorrsubstanshalt betydligt högre för plantor planterade i ung skog än i gammal skog, emedan specifik bladyta var signifikant lägre för plantor planterade i ung

skog jämfört med gammal skog. Rotisoleringsbehandling (med eller utan dike) hade ingen effekt på mellanarts-specifika egenskaper.

Korrelationen mellan inter-specifika egenskaper och planttillväxt var signifikant för de flesta av egenskaperna som analyserades. Planthöjd och rothalsdiameter under de två första åren efter plantering var signifikant- och positivt korrelerat med bladtjocklek och negativt med specifik bladyta. Den nuvarande årliga tillväxten i höjd (CAIH) och rothalsdiameter (CAID) var positivt korrelerad med bladdensitet och bladtorrsubstanshalt men negativt korrelerad med specifik bladyta. Korrelationsanalys mellan intra-specifika egenskaper och planttillväxt utfördes också och visar på signifikant korrelation mellan bladtjocklek och stamtorrsubstanshalt med planttillväxt i tre av fyra arter, särskilt var bladtjocklek korrelerad med både diameter och höjdtillväxt av två av fyra arter.

Sammanfattningsvis belyser denna studie vikten av funktionella egenskaper vid analys av överlevnad och tillväxt av olika arter under olika miljöförhållanden. Detta nya egenskapsbaserade tillvägagångssätt kan förutsäga överlevnad och tillväxt och därmed användas som vägledning för framtida restaureringsprojekt under liknande klimat- och miljöförhållanden. Eftersom storleken på den aktuella studien var i minsta laget, rekommenderar jag ytterligare forskning med större prov-storlekar för varje behandling samt flera arter för att på så sätt kunna identifiera de bästa prediktiva egenskaperna.

Nyckelord: Funktionella egenskaper, bladegenskaper, restaurering, stamegenskaper, tropiska torrskogar,

Preface

This is a master thesis on advanced level; it consists of 30 hp and is the final work of my education: MSc in forest sciences, on Jägmästarprogrammet given at SLU, The Swedish university of Agricultural Sciences. The work has been done for the Department of the Southern Swedish forest research Centre in Alnarp. I did a Minor Field Study in Costa Rica were I helped a PhD student, Geraldine Derroire, with her experiment. The study was done in collaboration with Sida and SLU and the fieldwork was carried out in Costa Rica during the summer 2015.

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Last but not least I want to give a huge thank to my family, and all friends that has supported me through all of this, without you I wouldn't have been where I am today.

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1. Introduction

1.1 General background

Many of the seasonally dry tropical forests (SDTF) in Central America has been destroyed due to land use change during the 1930-70s and only 1.7% remains in its natural stage (Calvo-Alvarado *et al.*, 2009). Forests have been harvested for timber production and cleared for cattle ranging, and the land has been degraded. In recent years people have put more effort into restoration of the forests, trying to reforest the areas again (Griscom & Ashton, 2011). In for example Guanacaste in the northwest part of Costa Rica, cattle farming has been very common, but since the 1970s when the market for cattle was reduced, people started to convert rangeland back to forest (Calvo-Alvarado *et al.*, 2009), and now many restoration project are going on in the region (ACG, http://www.acguanacaste.ac.cr/).

Deforestation and forest degradation are big concerns around the world, resulting in biodiversity loss, soil degradation, erosion and greenhouse gas emissions. This has not only consequences for the nature but also for the indigenous forest-dependent communities in the area, who are dependent on the resources. Forest landscape restoration (FLR) has therefore gained global attention as a triple win solution, by restoring forest landscapes, enhancing human well-being in deforested or degraded landscapes and making the landscape more resilience to climate change. To this end, the Global Partnership on Forest Landscape Restoration has been initiated to address the "Bonne Challenge" (http://www.forestlandscaperestoration.org/;), aiming at restoring 150 million hectares of lost forests and degraded lands worldwide by 2020.

Forest restoration, defined as the process of assisting the recovery of degraded, damaged, or destroyed forest ecosystem, is an intentional activity that initiates or accelerates the recovery of a forest ecosystem with respect to its health, integrity, and sustainability (SER, 2004). It encompasses a flexible package of site-based methods; ranging from natural regeneration (passive restoration) to establishment of forests by seedling planting or direct seeding (active restoration). In practice, it is not always straightforward to decide the best restoration strategy for a particular area because each area has its own disturbance history, degree of resilience, reference information, and surrounding landscape, as well as the applicable legal and socio-economic background. Therefore, it is essential to identify the most suitable strategy, aiming at reducing costs, time and enhancing restoration effectiveness. Generally, the cost of restoration increase with increasing level of intervention from natural regeneration to restoration planting, which in turn depends on the extent of degradation (Stanturf *et al.*, 2001). Thus, the choice of suitable species and cost-effective restoration methods are critical in any restoration projects. Many restoration projects in the past have recorded limited success. One of the main reasons is the difficulties in selection of species

with appropriate characteristics to fit into the prevailing environmental conditions. Thus, an efficient approach to species selection for restoration planting is necessary for restoration practices to be successful. Trait-based approach could be one possibility to reach success.

1.2. Plant functional traits

A trait is defined as "morphological, anatomical, biochemical, physiological and/or phenological features measurable at the individual level" (Violle *et al.*, 2007). A functional trait is "any trait which impacts fitness indirectly by its effects on growth, reproduction and survival" (Violle *et al.*, 2007). A variety of plant functional traits has been proposed to be of importance for understanding plant regeneration and performance (Garnier & Navas, 2011; Laughlin *et al.*, 2010; Wright *et al.*, 2004). The most common functional traits include Leaf-Height-Seed traits, which are related to the leaf economics spectrum (Wright *et al.*, 2004) and reproductive strategies (Laughlin *et al.*, 2010). Leaf functional traits include specific leaf area, leaf dry matter content, leaf density, and leaf thickness, of which specific leaf area and leaf dry matter content are highly related with the leaf economics spectrum (Garnier & Navas, 2011). The various leaf traits represents trade-off between strategic allocations to construction costs (Wright et al., 2006), photosynthetic rates, leaf life spans, and related to environmental or nutrient stresses and disturbances, plant–herbivore interactions, litter decomposition, and nutrient cycling (Souza *et al.*, 2015; Martinez-Garza *et al.*, 2013; Poorter & Bongers, 2006).

Total height and reproductive height play a functional role in resource acquisition and reproduction. Tall individuals have a competitive advantage over short ones in accessing light (Ruger *et al.*, 2012); they tend to be deep-rooted (Violle *et al.*, 2009) and hence they are likely to have access to larger water sources during the dry season than smaller, shallow-rooted plants. Reproductive height, at which flowers and seeds are produced, facilitates pollination and seed dispersal (Kuparinen, 2006). According to the global wood economics spectrum (Chave *et al.*, 2009), wood density relates to the trade-off between plant growth rates and nutrient transport, support and stem defenses against pathogens, herbivores, or physical damage by abiotic factors, while wood nutrient content relates with allocation pattern.

Functional traits have been a hot topic in community ecology and the understanding of plant life history strategies (Alder, 2014). Trait-based approach is widely used in a broad scale, from individual to ecosystem level; from stand- to global scale, to understand plant strategies for acquisition or conservation of resources and responses of plant to environmental conditions. Studying functional trait at the community scale provides information on how the community assembles. For instance, in a study made in a dry tropical forest in Costa Rica (Hulshof & Swenson, 2010), leaf trait variation within and across ten co-existing tree species

were examined, and the results showed that the majority of variation in traits was explained by between species differences. Simple-leaved species followed a trend across all leaf traits sampled with high between-species variation, followed by within-species variation, and lastly variation between leaves of separate individuals. Compound-leaved species followed this trend when individual leaflet trait values were not included than variation for compound-leaved species that was found to be highest between species and between leaflets. The study also showed that it is difficult to differentiate between species on the basis of functional traits even when the sampling size is big; a sample size of at least 10 is suggested when calculating mean trait values for individual species for entire communities. In species rich communities like tropical forest communities, the sample size may need to be even larger, which makes generating functional trait data sets more challenging. It remains unclear if community level trait values will allow comparisons on a larger geographic scale or if species traits are generally similar across scales (Hulshof & Swenson, 2010).

Most previous studies have used average trait values per species, assuming that the intra-specific (within species) variability of a trait is very low compared to inter-specific (between species) variability. Recent studies, however, challenge this assumption and recommend taking intra-specific variability into account (Violle *et al.*, 2012). This variability can result from genetic variability or from individual plastic response to environmental conditions in trait-based studies to improve the understanding of community assembly and how plant communities drive ecosystem processes (Violle et al., 2012; Kumordzi, 2015). For instance, Kumordzi (2015) found significant within-species trait variability among dominant species, and that within-species trait variability was highly responsive to differences in environmental conditions among ecosystems, in this case different island sizes. Also across contrasting environments, within-species trait variability sometimes explained a greater amount of variation in overall community-level responses than between-species trait variability. It has been shown that specific leaf area (SLA) was low and leaf dry matter content (LDMC) was high for *Betula pubescens* growing on nutrient limited small islands compared to nutrient richer large islands, indicating that leafs on trees growing on small islands conserve nutrient whereas on large islands acquire nutrients (Kumordzi, 2015). This study also provides insights on how changes in resource availability drive community trait composition, species co-existence and consequently community response.

Water availability is the most limiting factor for the survival and growth of tree species in seasonally dry tropical forests. More than 80% of the annual precipitation occur during 5 months (the wet season), and it rains more than 100 mm per month during this period. Meanwhile, during the dry season it rains only 10 mm per month. Inter-annual rainfall variability is also common in SDTF, for example due to El Niño years, which makes it drier than usual in some years (Dirzo *et al.*, 2011). There was an El Niño phenomenon in Guanacaste, Costa Rica during the study period in 2015, resulting in very dry conditions in northwest part of Costa Rica. Species native to such environments have developed different

adaptations to withstand, avoid or tolerate water limitations. Drought-tolerant species (evergreen species) delay drought stress by maximizing their access to water, whereas minimizing transpiration water loss, and resource cavitation, and by having high biomass investment to the root system, (high specific root length, small leaf area, and strong stomatal control). Whereas drought-avoiding species (deciduous species) maximize resource capture during a limited growing season and avoid stress in the dry season by shedding and drop of their leaves (Markesteijn & Poorter, 2009).

Dry forest species tend to have compound leaves with high SLA and taproots (drought avoiders), high stem dry matter content (SDMC) and low leaf area ratio (drought resisters), suggesting that reduction of transpiration and avoidance of xylem cavitation are important for their success (Poorter & Markesteijn, 2008). A study made by (Markesteijn & Poorter, 2009) showed that deciduous species have high specific leaf area (SLA) and a large biomass while evergreen species have greater stem density (SD) and a large root system with a high total root length. Species in dry arid climates also invest in thicker leaves as an adaption to water stress and therefore both tolerate shade and drought (Reich *et al.*, 2003). Other authors found that LDMC is negatively correlated with SLA and leaf thickness and positively correlated to SD (Castellanos Castro & Newton, 2015; Pérez-Harguindeguy *et al.*, 2013; Cornelissen *et al.*, 2003).

Light and nutrients are also resources essential for species survival and growth. Competition for resources can be a stress factor for plants and are important for the distribution of species as well as their evolution and adaptation (Craine *et al.*, 2013). Generally, early successional species are more light-demanding than late successional species. Therefore it is important to consider light availability when selecting species during succession. Drought tolerance is believed to be more or less of the same pattern in early- and late successional species, but the latter is more succumb to increased mortality in dry years, most likely due to lack of drought-avoiding strategies like deep rooting systems, and water storage in roots and stems (Schönbeck *et al.*, 2015).

It was shown that dry forest species had a longer drought survival time, 62 days compared to 25 days for moist forest species (Poorter & Markesteijn, 2008). The same study claimed that deciduousness explained 69% of inter-specific variation in drought survival, and among evergreen species (drought resisters) stem density explained 20% of the drought survival. Thus, compound leaf and dry matter content are positively correlated to drought index. Tree species differ in their survival and growth strategies under different environmental conditions; for example, plants in arid climates invest in production of thicker leaves as an adaption to water stress. The tree species with thicker leaves are tolerant to both shade and drought (Reich *et al.*, 2003). In general it seems like evergreen tree species have thicker leaves than deciduous species (Krober *et al.*, 2015).

According to the leaf economical spectrum, there are two ends of the spectrum (Wright *et al.*, 2004; Reich *et al.*, 2003) where the one end is with quick return on investment (use of resources) and the other end is with slow return on investment (conservation of resources). Acquisitive species use the resources under high light or water availability conditions whereas conservative species provide tolerance to both shade and drought (Castellanos Castro & Newton, 2015). As a result conservative species are characterized as shade tolerance and slow growth while that of acquisitive species are shade intolerance and fast growth. Deciduous species are more acquisitive users of resources with higher SLA but lower LDMC and SD than evergreen species (Pérez-Harguindeguy *et al.*, 2013). One example of deciduous species are those in the family *Fabaceae*, which are legumes often with compound-leaves, and with high leaf nitrogen, high stem density (SD), but lower SLA, which is in contrast to the assumption above. Legumes are common in seasonal dry tropical forests and are drought avoiders (Powers & Tiffin, 2010).

Tissue density is the ratio between dry mass and volume and is a key functional trait, associated with many critical aspects of plant growth and survival and is an important predictor of plant strategies. With less dense tissues the plant gets a fast relative growth rate and a rapid resource acquisition, as the plant rapidly can expand leaf, stem or root system with a low investment on dry matter. But the produced soft tissues for fast growing species tends to have a shorter life span and is also usually more vulnerable to herbivory and pathogens than the highly dense tissues typical of slow-growing species (Birouste *et al.*, 2013).

1.3. Applications of trait-based approaches in forest restoration

Functional trait analysis across plant species and environments is a rapidly developing research field with many possible applications for forest restoration practice. Recently, trait-based approaches have been applied for predicting demographic rates (Poorter *et al.*, 2008) and restoration success (Asanok *et al.*, 2013; Martinez-Garza *et al.*, 2013), for suitability scoring of tree species for direct sowing (Tunjai & Elliott, 2012) and for predicting drought performance and distribution of Mediterranean woody species (Lopez-Iglesias *et al.*, 2014). Based on four functional traits (seed volume, specific leaf area, wood density, and adult stature) and two demographic attributes (diameter growth and tree mortality) for large trees of 240 tree species from five Neotropical forests, Poorter et al. (2008) demonstrated that morphological traits explained 41% of the variation in growth rate and 54% of the variation in mortality rate, with wood density being the best predictor of demographic rates. In a restoration planting in abandoned pasture in Mexico, (Martinez-Garza *et al.*, 2013) evaluated the relationship between performance (survival and growth) of 24 species and 13 underlying functional traits that are important for leaf display, tree architecture and reproduction. The findings from this study demonstrated that a positive relationship between growth rates and survival and crown size, and negative relation to seed mass, and no relationship to leaf traits.

The authors concluded that both pioneer and non-pioneer species with small seeds and large crown length have the best mid-term performance and are good candidates for restoration plantings in pastures. In the tropical mountain forests of northern Thailand, Asanok et al. (2013) found a significant correlation between dominance of certain species in primary forests with wood density and seed size; and suggested that the restoration of primary forests by natural regeneration is challenging but the success could be partly predicted by their functional traits.

A study made in a semi-evergreen tropical moist forest in Bolivia by Poorter & Bongers (2006) provides further evidence about the importance of leaf traits in predicting plant performance both in gaps and in forest understory. It was found that species with high growth rates in gaps (light demanding species) had cheap, short-lived, and physiologically active leaves; whereas species with high survival in understory (shade tolerant species) formed long-lived well protected leaves (leading to reduction in biomass loss). A trade-off was found between growth and survival, where species with long-lived leaves (high survival) had low SLA whereas species with fast growth rates were characterized by a high SLA and nitrogen concentration, and were physiologically more active by having high photosynthesis, respiration, and conductance rates (Poorter & Bongers, 2006). This means that short-lived species that grows faster will also die faster in this area (Poorter & Bongers, 2006). For Mediterranean woody species, functional traits predict drought performance and distribution of species in this ecosystem. In their study, Lopez-Iglesias et al. (2014) found a continuum of drought among 10 species, ranging between two contrasting species functional extremes. In one end of the continuum are acquisitive fast-growing deciduous species with thin, soft metabolically active leaves, with high resource use and vulnerability to drought and on the opposite end of the continuum are conservative slow-growing evergreen species with sclerophyllous leaves, deep roots, a low transpiring area, and low water use, resulting in high drought survival and drought tolerance.

All these studies show that trait-based approach has great potential as decision-tool for forest restoration but more studies are needed to find out the best predictive traits. The recent study by Paine et al. (2015) concluded that functional traits alone are weak predictors of juvenile tree growth at a global scale; i.e. there are no one-size-fits-all traits that can be used across all environments, but they rather recommended to determining functional traits that predict plant performance under specific environmental conditions. In view of this gap of knowledge and the fact that tropical dry forests are much less studied than tropical moist and temperate forests, although being the most threatened ecosystems on Earth, this study was conducted in the dry forests of Costa Rica with the following.

2. Objectives and research questions

The main objective of this study was to examine whether leaf functional traits explain establishment of seedlings planted under different environmental conditions, and hence develop directives for selection of restoration species based on functional traits in the future. Specifically, the proposed study investigated (1) the relative importance of inter-specific and intra-specific variability of functional traits in explaining difference in seedling growth and survival under different environmental conditions, and (2) the possibility to identify ecological and environmental drivers of this inter- and intra-specific trait variability.

The specific research questions and the corresponding hypotheses of the study were:

- Do intra- and inter-specific traits of planted seedlings vary between successional stages of forest (between old and young forests) and trenching treatment (with versus without)?

  Hypothesis: Intra-specific traits are expected to be higher in resource limited environment; and species are expected to differ in functional traits in response to resource limited environments.

- Do survival and growth of planted seedlings vary between successional stages of forest (between old and young forests) and trenching treatment (with versus without)?

  Hypothesis: Survival and growth are expected to vary in response to above and below ground resources (light, nutrient and soil moisture).

- Can functional traits predict survival and growth of planted seedlings?

  Hypothesis: Significant relations is expected between traits and field performance of seedlings

3. Materials and Methods

3.1. Study site

The study was conducted in a seasonally dry tropical forests (SDTF), situated in a flat alluvial lowland area with fertile soils (Calvo-Alvarado *et al.*, 2009) in the Conservation Area of Guanacaste (ACG), more precisely in the forest experimental station Horizontes, in the Northwest part of Costa Rica (Figure 1). The Sector Santa Rosa, Area de Conservation Guanacaste is located between 10°48´53´N and 85°36´54´ W (Hulshof & Swenson, 2010). The altitude in the area ranges from 60 to 184 meter above sea level, the mean annual temperature is 28 C° and the annual precipitation range from 1500-2000 mm per year (ACG, Programa de Restauracion y Silvicultura, 2012).

Guanacaste is an area where much of the original and existing tropical dry forests of the country are located. In the past, the land cover was a mix of natural savanna and tropical dry forest. The land use pressure was high in the mid-1800s to 1970s due to beef production and timber products which were of major economic importance. The deforestation had its peak in the 1950-1970s when beef production expanded (Calvo-Alvarado *et al.*, 2009). Following the decline in international beef prices in the 1980s, the beef production decreased in the area (Calvo-Alvarado *et al.*, 2009) while interests in forest protection increased.

A restoration project were started, and a network of protected areas, the conservation area of Guanacaste (ACG), was established, where they tried to reforest the area naturally by fire control (Calvo-Alvarado *et al.*, 2009). One of these areas is Horizontes, which was established by a donation of 7000 ha to ACG in 1987 (http://www.acguanacaste.ac.cr/acg/historia). Horizontes was a former pasture land, used for cattle ranching and crop farming, but later on converted to experimental plantations for restoration and silviculture; and most of the area is monoculture and mixed-species plantations with different planting designs. The station is nowadays a mosaic of secondary forests of different ages. Global forest watch showed an increase in tree cover of Horizontes between 2001-2012 (www.globalforestwatch.org) and today 70% of the area is covered by forest (ACG, Programa de Restauracion y Silvicultura, 2012).

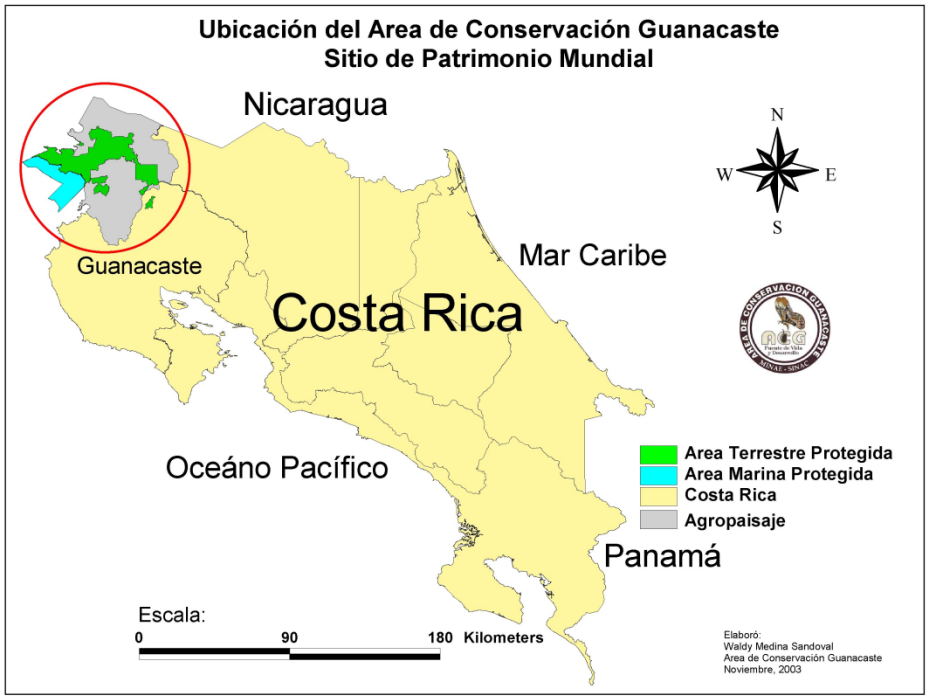


Figure 1. *Location of the Conservation Area of Guanacaste (map produced by ACG)*

3.2. Experimental settings

The study was built on an experimental setting established by Geraldine Derroire in May 2013, which included planting of about 800, three to four month old seedlings of six different tree species in two different secondary forests, Bajo Sombra, 30 years old and Pista Aterizaje, 15 years old. There were four plots in each site. Two treatments were applied in a full factorial design; with or without litter removal and with or without trenching to exclude root interactions (Figure 2). The trench was 50 cm deep and 20 cm wide and made by two layers of plastic sheets. Each treatment plot had four replications to which four seedlings of each of the six species were planted randomly at 50 cm spacing, by using a random number generator to indicate their coordinates. The following species were used: *Astronium graveolens* (RonRon), *Cochlospermum vitifolium* (PoroPoro), *Dalbergia retusa* (Cocobolo), *Hymenaea courbaril* (Guapinol), *Simarouba glauca* (Aceituno), *Thouinidium decandrum* (Sardino). The species were selected amongst the common species in the area, their ecological description and growth habits are presented in Table 1. The seeds were collected in the field and raised in a nursery by experienced staff at the research station.

Table 1. Natural distribution, growth habit and some ecological characteristics of the species used in the study. Information taken from (Croat, 1978; AdC, 2012)

Species	Natural distribution	Growth habit and ecology
Astronium graveolens (RonRon)	Occurs in primary, secondary, wet and dry forests and shrub land from Mexico to Brazil, Bolivia & Paraguay; Occurs in areas at altitudes ranging from sea level to 1500 m with 750-3500 mm annual mean rainfall, 20-35 °C annual mean temperature and 3-6 months dry season; Adapted to different sites, from flat to moderate slopes, from fertile to rocky and poorly drained alluvial soils, best developed in areas of moderate and well drained sandy soils.	Long-lived, slow growing commercial hard wood species reaching 35 m in height, 1.0 m in dbh, 4.1-7.9 m^3/ha/yr in MAI and 30 years rotation (wood density = 0.85-1.28 g/cm^3); Straight stem with round crown with low, irregular and up branches; Has alternate, odd-pinnate compound leaves (4-15 pairs of leaflets) that are deciduous during the dry season; Intermediate-sized seeds (1-1.5 cm) with good natural regeneration; Used in enrichment and restoration planting and for plantation (3 × 3 m) for biodiversity and economic purposes due to high survival rates
Cochlospermum vitifolium (Poroporo)	Distributed from Mexico to South America; Most common in young pastures, secondary forests and disturbed areas.	Small to medium-sized fast growing pioneer species with 3-12 m in height, 10 cm in dbh with very soft wood; Leaves are palmate, simple and alternate; Possess small seeds (less than 1 cm).
Dalbergia retusa (Cocobolo)	Distributed from Mexico to Panama, in a wide range of climates, from dry to dry mountain to wet forests, from altitudes from sea level up to 1200 m; Occurs in areas with 2000 mm annual rainfall, 25-35 °C mean temperature and ca. 3 months dry season.	Slow growing hardwood species up to 15-20 m in height, 40-70 cm in diameter; 9 m^3/ha/year in MAI; 0.99-1.22 g/cm^3 in wood density; Has compound leaves with 7-15 leaflets; Grows well in open areas, less dense forest, with deep to rocky soil and free draining & nitrogen fixing Seedlings tolerant to light but natural regeneration scarce within forests.

Species	Natural distribution	Growth habit and ecology
Hymenaea courbaril (Guapinol)	Occurs in dry to humid forests in Southern Mexico, Central America, to Peru, Bolivia, Brazil and Guyana; Growing along riparian forests or evergreen groves on flat to steep slopes at altitudes from near sea level to 1300 m, annual precipitation of 800-4000 mm, average temperature 20-30°C, dry season of 0-6 months; It grows best on deep sandy soils, fertile and well drained, with pH 4.8-6.8.	Slow growing hard wood tree reaching up to 40 m height, 100 cm diameter with wood density of 0.7-0.89 g/cm^3; Light-demanding species with umbrella-shaped crown with compound alternate leaves with two leaflets; Works well in plantations with weed control at younger stage, used as shade tree, resistant to root fungi; Natural regeneration poor, not good in swamps or shade, and not a good competitor with weeds.
Simarouba glauca (Aceituno)	Native from Florida, now distributed tropical and subtropical wetlands of Central America, Mexico and the Caribbean; Grows in areas with altitudes ranging 0-800 m, slopes less than 30, annual average precipitation more than 1200 mm, average temperature 22-29°C and 0-6 months dry season.	Fast growing, shade tolerant, sub-canopy species reaching 25-27 m in height, 40-50 cm in dbh, 0.38 g/cm^3 in wood density with a rotation period of 45-65 years; Leaves are alternate, even-pinnately compound and seeds are large (over 1.5 cm); Adapted to a variety of soil types, from calcareous soils, rocky mountain slopes to more superficial deep floodplains and streams; Used in enrichment planting and ecological restoration of natural forests, but perform poorly in very disturbed sites with precipitation less than 1200 mm/year.
Thouinidium decandrum (Sardino)	Distributed from Mexico to Costa Rica at altitudes up to 1500 m in areas with 2500 mm/year annual precipitation, 26°C mean temperature and 3-6 months of dry season and tolerate poor soils with pH 6-7; Often found in dry forests along river and stream banks, but sometimes in the wet forest.	Evergreen medium sized tree species reaching up to 25 m in height and 40 cm dbh; Leaves are compounded, odd-pinnate, alternate and have very narrow lanceolate leaflets (opposite or alternate); Branches rising sharply and develop a broad crown; Hardwood species with high natural durability; Possess large seeds (over 1.5 cm).

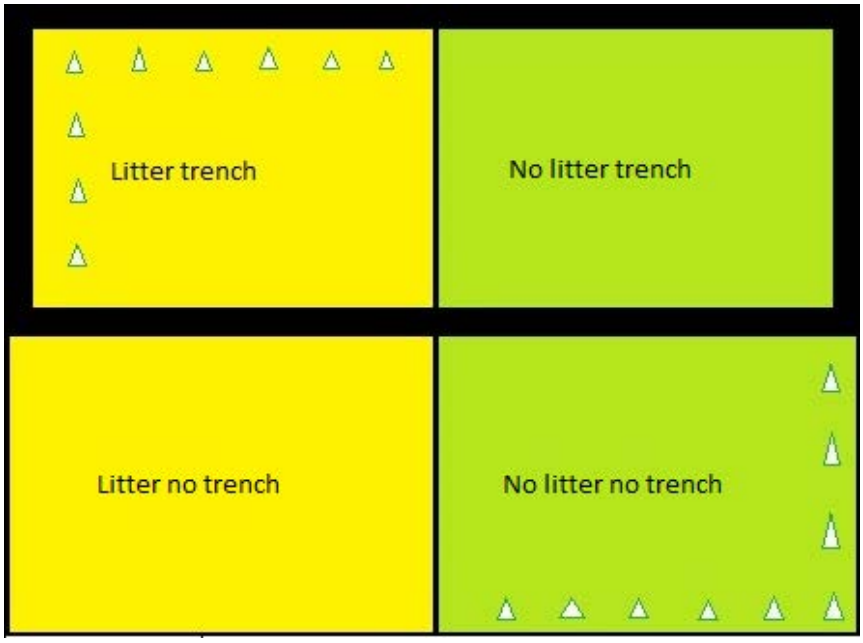


Figure 2. Experimental plot with all treatments. In each plot four seedlings of each 6 species were planted randomly (24 seedlings/ sub-plot).

3.3. Survival and growth assessment

The seedlings in each replicate plot were given an ID number, their coordinates registered, and survival and growth assessments were done in order of the sub-plots and seedling ID numbers. Survival, height and root collar diameter of all planted seedlings were assessed every six months. The survival status of each seedling was recorded by observation, such that a seedling was considered dead if it was defoliated and/ or the stem was dried. The root collar diameter was measured at the base of each seedling at ground level with digital calliper, and the height of the main shoot was measured from the ground to the terminal bud with measuring-tape. For this thesis work, seedling height and diameter data from July 2014 (after 1 year) and July 2015 (after two years) were used to calculate growth current annual increment of the seedlings that was still alive in July 2015.

3.4. Measurements of functional traits

In this study, six different functional traits associated with resource acquisition were measured following the standardized protocol for measurement of plant functional traits (Pérez-Harguindeguy *et al.*, 2013; Cornelissen *et al.*, 2003). The measured functional traits were specific leaf area (SLA), leaf dry matter content (LDMC), leaf density (LD), leaf thickness (LT), stem dry matter content (SDMC), and stem specific density (SSD). First, survival assessments and reconnaissance survey were made to determine possible candidate seedlings for the trait measurements, and then two seedlings with two healthy leaves per species in each

replicate sub-plot were chosen (if it was possible). If there were more than two healthy seedlings of the same species in a replicate sub-plot, the samples were selected by computer generated random number. The maximum number of samples per forest type was 48 (2 seedlings × 6 species × 2 trenching treatments × 2 litter treatments). The selected seedlings were carefully dug out one by one, with the base of the stem marked with black marker (to be able to differentiate the above and below ground parts). The harvested seedlings were immediately cut at the marked stem base and placed in tubes filled with distilled water and then stored in a cooler (ice-box with ice-cubes) to avoid rapid desiccation of the above- and below-ground organs in the field. Each tube was labeled with seedling ID number. After transporting the samples to the laboratory, the measurements were started as soon as possible.

For leaf trait measurements, 3 non-damaged, fully expanded leaves, as evenly distributed along the stem as possible, were taken from each seedling if possible. Some samples did not have leaves at all. Each leaf was surface dried using absorbent paper and placed on labeled paper-bags per leaf. The fresh mass of each individual leaf was taken using Scout Pro Balance in gram (with a precision of three decimals). Leaf thickness in mm was measured at an intermediate position between the border and the midrib and between at the tip and the base of the leaf using digital micrometer (with a precision of three decimals). Care was taken to avoid major secondary veins during thickness measurement. Leaf area was measured using a flat-bedded scanner (Cano Scan Lide 210, equipped with a program called Canon MP Navigator EX 4.0) and saved in the format of jpg color photos (600 dpi). The leaf area was then calculated by a pixel counting software called imageJ, which converted pixels to cm^2. After all leaf measurements, the leaf samples in the paper-bag with the right ID were oven dried at 70°C for at least four days for determining the dry weight.

For stem trait analysis, samples were drawn from the tubes, surface-dried with absorbent paper and 10 cm stem sections (between 5 and 15 cm of height) were prepared and placed on paper-bags labelled with seedling number. The fresh mass of stems was determined using the same balance as previously described. The length of stem sections was measured using sewing tape and/ or digital caliper (with a precision of three decimals). If the stem was bent, the length of stem section was measured following the bending. If the stem was in zig-zag sections, the length of each section was measured and added together. The stem diameter was measured with the digital caliper at 3 positions along the stem; at the base, in the middle and at the top and the average value was calculated. To determine the stem dry weight, the stem sections were placed in paper-bags, with right seedling ID, in a drying oven, at 70°C for at least six days. After drying, the dry mass of stem sections was taken using a balance, the same as previously described. Stem-specific density (SSD) is calculated as the oven-dry mass of a section of the stem divided by the fresh volume of the same section (units: mg/mm^3).

3.5. Data analysis

Prior to statistical analysis of the data, some derivative traits were calculated as follows. Leaf dry matter content was computed by dividing leaf dry biomass by leaf fresh biomass (mg/g); Specific leaf area was calculated by dividing leaf area with dry biomass (mm^2/mg); leaf density as dry mass divided by fresh volume (g/cm^3) assuming volume of a cone ($\Pi*h/3$ (R^2*Rr*r^2). Stem dry matter content was calculated as stem dry biomass divided by stem fresh biomass (mg/g) while stem specific density as dry biomass divided by fresh volume (mg/mm^3).

Initial data exploration showed some problems; such as missing values in some of the treatment plots (particularly litter removal treatment) and high mortality of *C. vitifolium* (PoroPoro) and *S. glauca* (Aceituno) seedlings. These problems made the original design unbalanced; therefore the final statistical analysis were made for four species in relation to forest type (old versus young) and trenching (with versus without) treatment where no litter removal was done. Thus, each forest type had a total number of 48 (6 species × 4 plots × 2 trenching treatments × 1 litter treatment) samples computed based on the mean values for each species in each treatment in each plot. Two-way analysis of variance (ANOVA) was performed to determine differences in intra-specific trait variabilities and growth of planted seedlings between forest types (old versus young) and trenching treatments (with versus without) using individual seedlings as replicates. Three-way ANOVA was performed to examine significant difference in measured traits, survival rate and growth of planted seedlings after two growing seasons in relation to species, forest types and trenching treatments based on plot means (inter-specific trait variabilities). ANOVA results were considered significant if $P < 0.05$ and to show tendencies if $0.05 < P < 0.10$. Means that exhibited significant differences were compared using Tukey's test at the 5% level. Pearson's Product-Moment Correlation was computed to examine the relationship between mean trait values of the four species and survival and growth rate of planted seedlings (correlation between inter-specific traits and seedling performance). Correlation analysis was also performed at individual seedling level for each species separately (correlation between intra-specific traits and seedling growth). All statistical analyses were performed using Minitab17 (Minitab Inc.).

4. Results

4.1. Intra-specific trait variabilities between forest types and trenching treatments

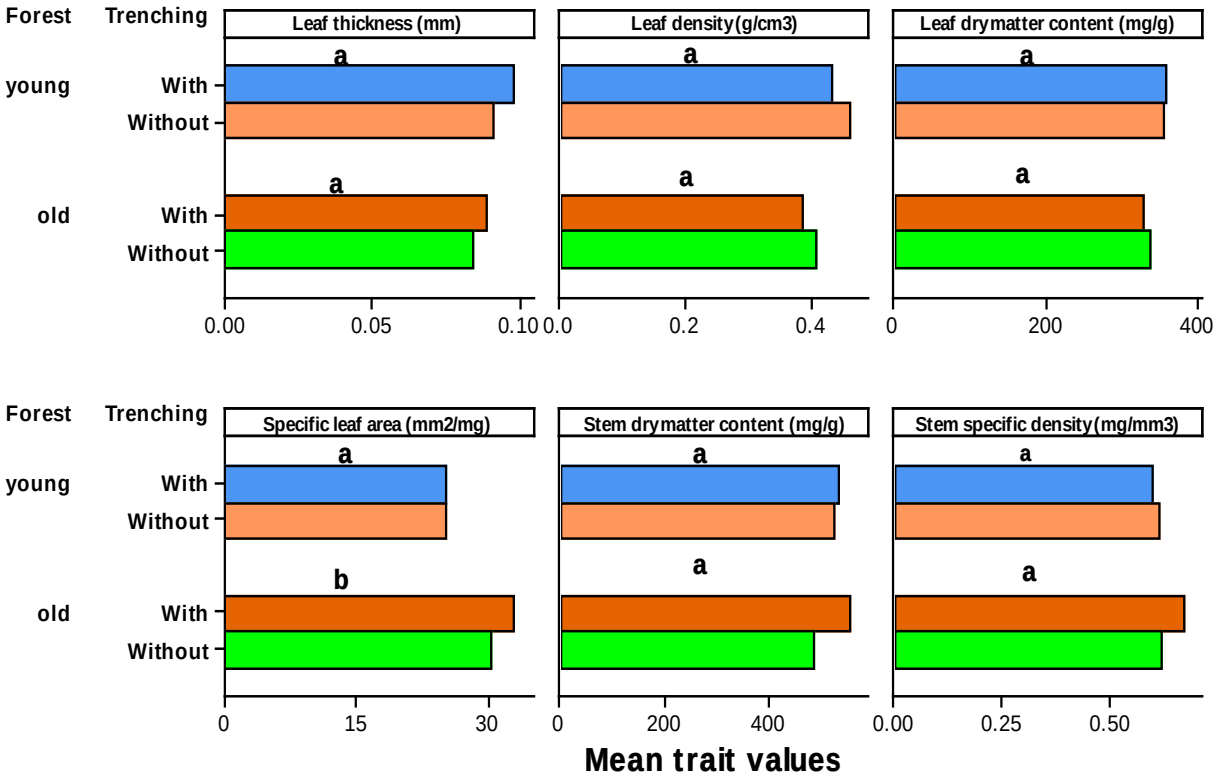
Intra-specific leaf and stem traits did not vary significantly between forest types and trenching treatment for all the studied species, except specific leaf area. Specific leaf area was significantly higher for seedlings of *A. graveolens* ($p = 0.015$) planted under old than young forests (Figure 3A). For *H. courbaril* seedlings, leaf thickness tended ($p = 0.089$) to be higher in young- than in old forest whereas specific leaf area ($p = 0.089$) and stem specific density ($p = 0.057$) tended to be higher in old- than young forests. For seedlings of *T. decandrum*, stem dry matter content tended ($p = 0.097$) to be higher in young- than old forest.

4.2. Inter-specific variabilities between forest types and trenching treatments

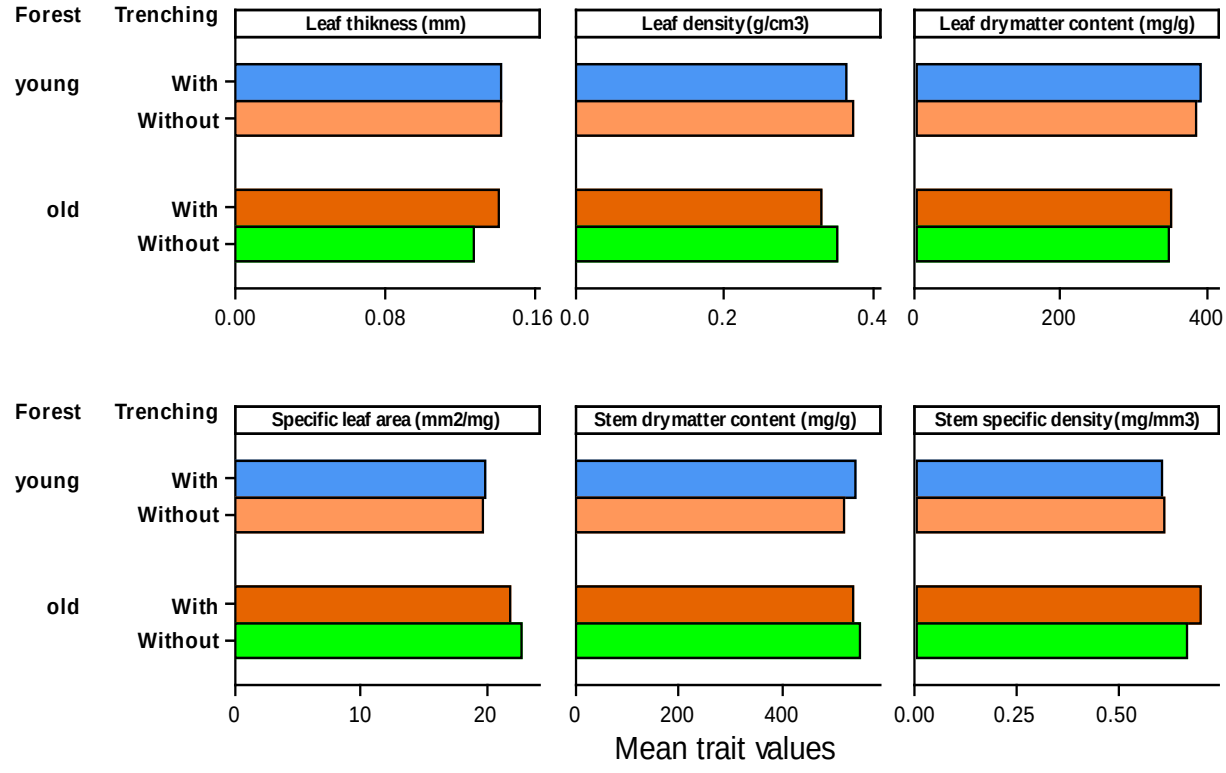
Three-way ANOVA showed highly significant differences in leaf- and stem traits among species ($p < 0.01$), except stem dry matter content ($p = 0.209$). Leaf thickness, leaf dry matter content and specific leaf area did also significantly vary between forest types ($p < 0.05$) but not between trenching treatments and interactions between species, forest type and trenching treatments. As the trenching treatment (with versus without) had no effect on inter-specific traits, results were presented for species and forest types combining the levels of trenching treatment (Figure 4).

The mean stem specific density, leaf dry matter content and leaf density were lower while specific leaf area was higher for *D. retusa* than other species (Figure 4). *H. courbaril* had higher leaf thickness than the rest of the species. For *A. graveolens* and *T. decandrum*, stem specific density, specific leaf area, leaf dry matter content and leaf density did not differ significantly. Among the traits analysed, leaf thickness and leaf dry matter content were significantly higher for seedlings planted in young than old forest; whereas specific leaf area was significantly lower for seedlings planted in young than old forest. The trenching treatment (with versus without) had no effect on inter-specific traits.

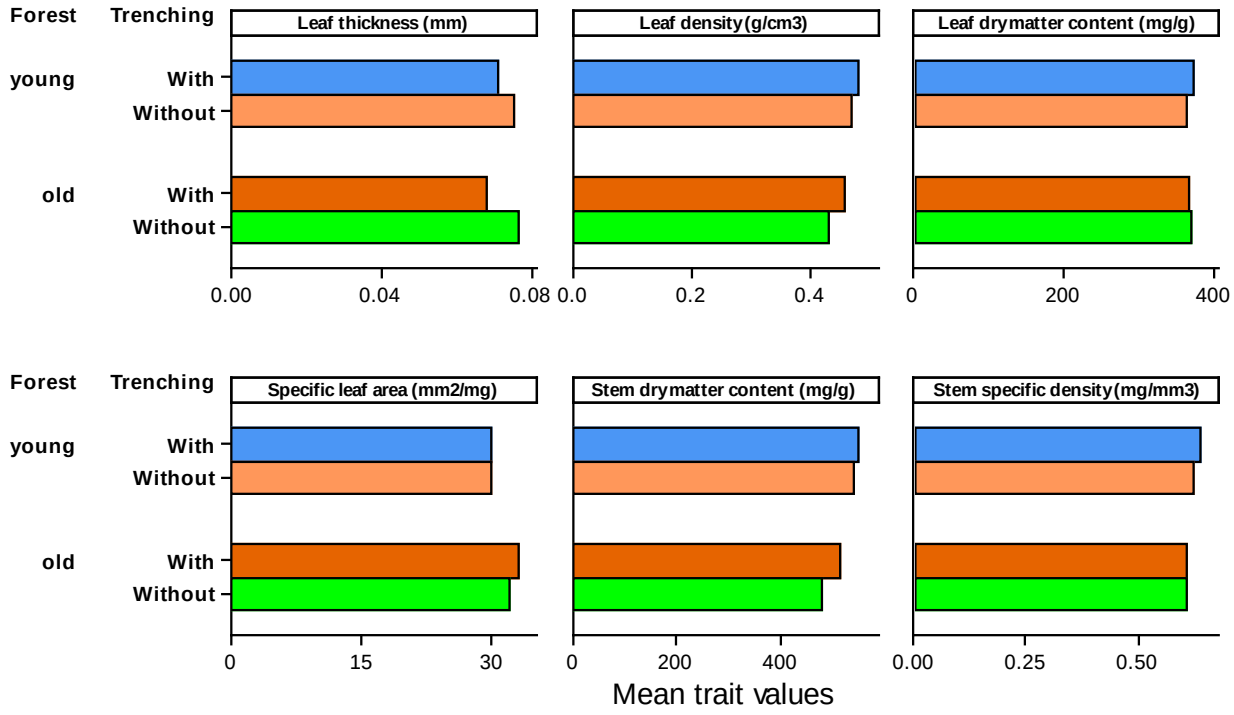
A) A. graveolens



B) H. courbari



C) T. decandrum



D) D. retusa

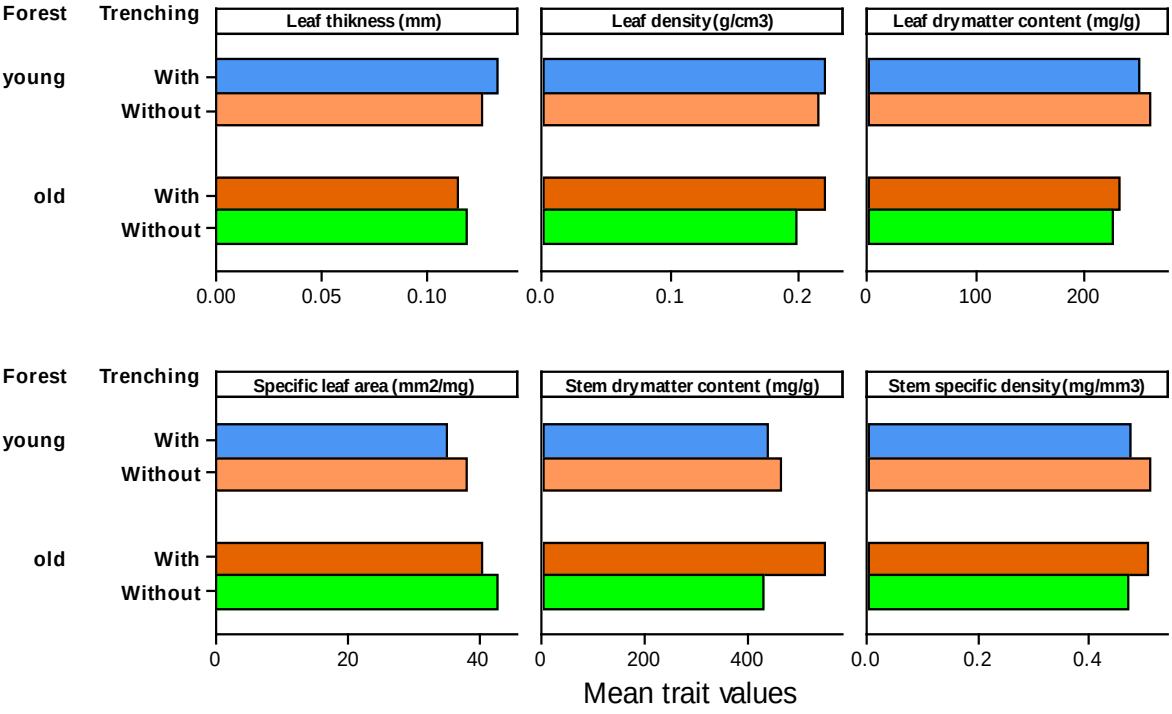


Figure 3. Intra-specific leaf- and stem trait variabilities across forest types and trenching treatment. Trait means followed by different letters exhibit significant differences between forest types ($p < 0.05$).

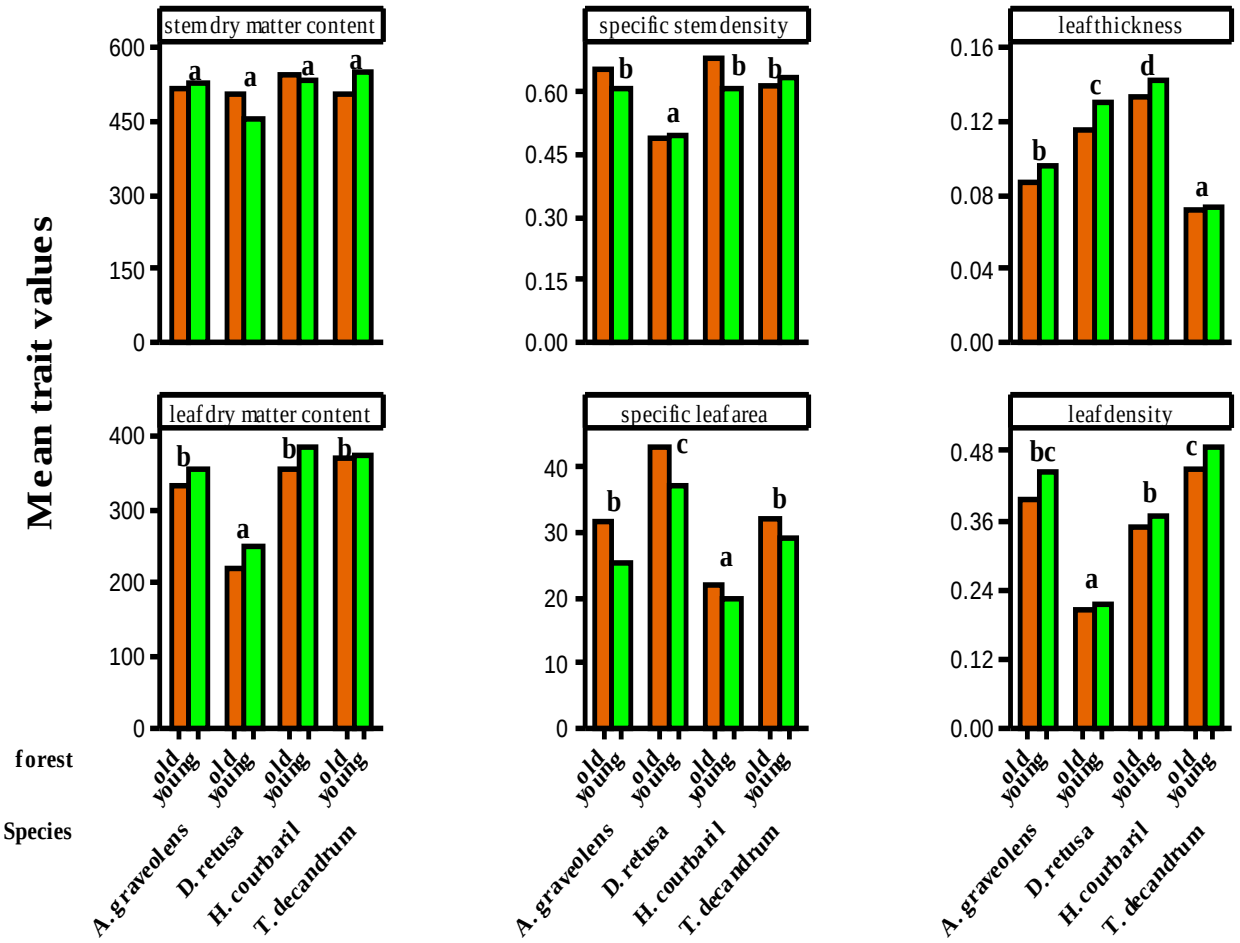


Figure 4. Inter-specific trait variability between forest types. Trait means followed by different letters exhibit significant differences among species ($p < 0.05$). Trenching treatment had no significant effect on any of the traits analyzed, thus the mean values are aggregated for forest types across all levels of trenching treatment.

4.3. Survival and growth of seedlings

Survival rate varied significantly among species ($p = 0.004$) and forest types ($p < 0.001$), but not between trenching treatments and the interactions between the three factors. Among species, *A. graveolens* had the highest survival rate compared with *D. retusa* and *T. decandrum* while *H. courbaril* seedlings had good survival rate (Table 2). For all species combined, survival rate was significantly higher for seedlings planted in young than old forests.

Table 2. Survival rate (%) of seedlings of four dry forest tree species planted in young and old forests with or without trenching (mean ± SE). Overall means for species and forest types across rows and columns, respectively followed by different letters are significantly different among species ($p < 0.05$).

	Forest types		Overall mean
Species	Old	Young	(species)
T. decandrum (Sardino)	59 ± 8	69 ± 6	64 ± 5[a]
D. retusa (Cocobolo)	54 ± 12	79 ± 8	67 ± 8[a]
H. courbaril (Guapinol)	38 ± 13	91 ± 7	73 ± 9[ab]
A. graveolens (Ronron)	75 ± 11	100 ± 0	88 ± 6[b]
Overall mean (forests)	60 ± 6[a]	85 ± 4 [b]	

Seedling height one year post-planting varied significantly among species ($p < 0.001$) and for the interaction between species and forest type ($p = 0.016$). The main effect of trenching treatment and its interaction with forest type and species were not significant ($p > 0.05$). One year post-planting, seedlings of *H. courbaril* was the tallest compared with *D. retusa* and *T. decandrum* but its height did not differ significantly from that of *A. graveolens* (Table 3). However, *A. graveolens* seedlings planted in young forest were taller than those planted in old forest. Seedlings of *T. decandrum* were consistently shorter in both young and old forests. As a whole, seedlings planted in young forest tended ($p = 0.062$) to be longer (mean = 29.85 cm) than those planted in old forest (mean = 23.75 cm).

Two-year post-planting, seedling height significantly varied among species ($p < 0.001$), forest type ($p = 0.040$) and their interaction (0.026), but not between trenching treatments (with versus without) and the interaction with species and forest types. Seedlings of *A. graveolens* and *H. courbaril* were taller than seedlings of *D. retusa* and *T. decandrum*; and seedlings were taller when planted in young than old forest (Table 3). This difference was mainly accounted to the significant difference in height of seedlings of *A. graveolens* between forests types; i.e. seedlings of this species were taller in young than old forest. For the rest of the species, differences in two-year post-planting height were not significantly different between young and old forests. The current annual height increment (CAIH) significantly varied with respect to species ($p < 0.001$) and forest types ($p = 0.046$) only. Seedlings of *A. graveolens* grew faster in height than the rest of the species, and height growth was twice higher in young than old forest (Table 4).

Table 3. Seedling height (cm) and its current annual increment (cm/yr) of four dry forest tree species planted in young and old forests with or without trenching (mean ± SE). Overall means followed by different letters within the columns are significantly different among species ($p < 0.05$).

Species	Forest type	Height-1	Height-2	CAIH
T. decandrum (Sardino)	Young	11.4 ± 0.5	12.7 ± 0.4	1.3 ± 0.3
	Old	10.0 ± 0.3	11.0 ± 0.4	1.0 ± 0.2
	Overall mean (species)	***10.7 ± 0.4^{a}***	***11.9 ± 0.4^{a}***	***1.2 ± 0.2^{a}***
D. retusa (Cocobolo)	Young	25.7 ± 3.4	28.3 ± 4.1	2.5 ± 1.1
	Old	23.8 ± 3.5	21.7 ± 3.4	-2.5 ± 3.2
	Overall mean (species)	***24.8 ± 2.3^{b}***	***25.0 ± 2.7^{a}***	***0.2 ± 1.8^{a}***
A. graveolens (Ronron)	Young	43.9 ± 7.7	66.3 ± 12.0	22.3 ± 4.5
	Old	20.3 ± 5.0	30.8 ± 9.1	10.4 ± 4.4
	Overall mean (species)	***32.1 ± 5.4^{bc}***	***48.5 ± 8.6^{b}***	***16.4 ± 3.4^{b}***
H. courbaril (Guapinol)	Young	38.3 ± 2.5	45.8 ± 3.3	7.5 ± 1.3
	Old	40.9 ± 5.4	48.1 ± 6.0	7.1 ± 2.1
	Overall mean (species)	***39.2 ± 2.3^{c}***	***46.6 ± 2.8^{b}***	***7.4 ± 1.1^{a}***

The root collar diameter one year post-planting varied significantly among species ($p < 0.001$), forest types ($p = 0.001$) and trenching ($p = 0.026$). There was a significant interaction effect ($p = 0.022$) of species and forest types on one year post-planting root collar diameter. Root collar diameter was bigger for *H. courbaril, A. graveolens* and *D. retusa* than *T. decandrum*; and it was bigger for seedlings planted in young than old forests, and in trenched than not trenched plots. The interaction effect of species by forest types was attributed to significantly bigger root collar diameter of *A. graveolens* seedlings planted in young than old forest.

Root collar diameter two-year post-planting varied significantly among species ($p < 0.001$) and between forest types ($p = 0.019$) while current annual diameter increment (CAID) only varied among species ($p < 0.001$). Seedlings of *A. graveolens* were two to three times bigger in root collar diameter two-year post-planting than *D. retusa* and *T. decandrum*, respectively while seedlings of *H. courbaril* were three times bigger in collar diameter than *T. decandrum* (Table 4). For all species combined, seedlings planted in young forests had bigger root collar diameter two-year post-planting than those planted in old forests. As a whole, *A. graveolens* was superior in collar diameter growth (CAID) followed by *H. courbaril, T. decandrum* and *D. retusa* (Table 4).

Table 4. Seedling root collar diameter (mm) and its current annual increment (mm/yr) of four dry forest tree species planted in young and old forests with or without trenching (mean ± SE). Overall means followed by different letters within the columns are significantly different among species ($p < 0.05$).

Species	Forest type	Diameter-1	Diameter-2	CAID
T. decandrum (Sardino)	Young	3.0 ± 0.2	3.0 ± 0.1	0.1 ± 0.1
	Old	2.4 ± 0.3	2.3 ± 0.3	-0.1 ± 0.2
	Overall mean (species	2.6 ± 0.2^{a}	2.7 ± 0.1^{a}	0.04 ± 0.1^{a}
D. retusa (Cocobolo)	Young	4.5 ± 0.4	4.7 ± 0.6	0.1 ± 0.1
	Old	3.5 ± 0.4	3.3 ± 0.6	-0.2 ± 0.5
	Overall mean (species)	4.0 ± 0.3^{b}	4.0 ± 0.4^{ab}	-0.04 ± 0.2^{a}
H. courbaril (Guapinol)	Young	4.9 ± 0.2	6.0 ± 0.5	1.1 ± 0.4
	Old	4.7 ± 0.8	5.9 ± 1.3	1.2 ± 0.5
	Overall mean (species)	4.8 ± 0.3^{b}	6.0 ± 0.5^{bc}	1.1 ± 0.3^{ab}
A. graveolens (Ronron)	Young	6.1 ± 0.8	8.9 ± 1.3	2.7 ± 0.6
	Old	3.1 ± 0.5	5.0 ± 1.3	1.8 ± 0.7
	Overall mean (species)	4.6 ± 0.6^{b}	6.9 ± 1.0^{c}	2.3 ± 0.5^{b}

4.4. Correlations between traits and field performance of planted seedlings

Correlation analysis on plot level revealed significant correlation between some of inter-specific traits and seedling growth (Table 5). Seedling height and root collar diameter during the first two years post-planting significantly and positively correlated with leaf thickness and negatively with specific leaf area. The current annual increment in height and root collar diameter were positively correlated with leaf density and leaf dry matter content but negatively correlated with specific leaf area.

Correlation analysis between intra-specific traits and seedling growth was also performed for each species based on individual seedling. Highly significant positive correlations between seedling growth (CAIH and CAID) and leaf traits were found for *A. graveolens*, except specific leaf area that were negatively correlated with both CAIH and CAID (Table 6). Significant negative correlations were found between CAIH and specific leaf area and stem dry matter content for *T. decandrum*. For *D. retusa*, CAIH and CAID were positively correlated with leaf thickness, while CAIH was negatively correlated with stem dry matter content. For *H. courbaril*, CAIH was positively correlated with leaf thickness, while CAID was positively correlated with stem dry matter content. As a whole, leaf thickness and stem dry matter content were correlated with seedling growth in three out of four species; especially leaf thickness was correlated with both diameter and height growth of two out of four species.

Table 5. Correlations between inter-specific leaf and stem traits and survival, height and root collard diameter after one and two years as well as current annual height increment (CAIH); and current annual root collar diameter increment (CAID) of seedlings of four TDF species. Lth, leaf thickness; LD, leaf density; LDMC, leaf dry matter content; SLA, specific leaf area; SDMC, stem dry matter content; SSD, stem specific density;

Growth			Leaf and stem traits			
Parameters	Lth	LD	LDMC	SLA	SDMC	SSD
Survival	0.138	0.137	0.221	-0.314*	-0.168	-0.209
Height-1	0.566**	-0.028	0.121	-0.506**	0.173	-0.022
Height-2	0.434**	0.115	0.215	-0.547**	0.076	0.022
CAIH	0.140	0.322*	0.324*	-0.507**	-0.094	0.087
Diameter-1	0.480**	0.027	0.135	-0.483**	0.154	-0.075
Diameter-2	0.366**	0.162	0.240	-0.541**	0.080	0.016
CAID	0.166	0.283*	0.313*	-0.503**	-0.020	0.117

* significant ($p < 0.05$); ** highly significant ($p < 0.01$)

Table 6. Correlations between seedling growth and intra-specific leaf and stem traits of four TDF species. Lth, leaf thickness; LD, leaf density; LDMC, leaf dry matter content; SLA, specific leaf area; SDMC, stem dry matter content; SSD, stem specific density; CAIH, current annual height increment; CAID, current annual root collar diameter increment.

Growth			Leaf and stem traits			
Parameters	Lth	LD	LDMC	SLA	SDMC	SSD
CAIH[1]	0.514**	0.509**	0.637**	-0.803**	-0.035	-0.234
CAID[1]	0.537**	0.439*	0.641**	-0.742**	0.048	-0.160
CAIH[2]	0.311	0.281	0.328	-0.458*	-0.364*	-0.192
CAID[2]	-0.040	0.233	0.169	-0.179	-0.111	0.072
CAIH[3]	0.544*	-0.032	0.296	-0.331	-0.563**	-0.098
CAID[3]	0.489*	0.119	0.437	-0.411	-0.406	0.166
CAIH[4]	0.483*	-0.304	-0.077	0.059	-0.182	0.012
CAID[4]	-0.052	0.155	0.281	-0.182	0.569**	0.005

1 = *A. graveolens* (Ronron); 2 = *T. decandrum* (Sardino); 3 = *D. retusa* (Cocobolo); 4 = *H. courbaril* (Guapinol); * significant ($p < 0.05$); ** highly significant ($p < 0.01$)

5. Discussion

5.1 Do intra-specific traits vary between forest types and trenching treatment?

The results show lack of significant differences in most of the intra-specific leaf- and stem traits between different environmental conditions. The reason for this non-significant result could be the small sample size used in the study. (Hulshof & Swenson, 2010) have shown that sample sizes of at least 10 replicates for each condition are needed for a good prediction of intra-specific variability. Kumordzi (2015) also looked on three dominant species; *Betula pubescens*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*; common birch, blueberry, and lingonberry on islands with different environmental conditions, small and big islands with low versus high nutrient availability due to fire frequency (more frequent on big islands → more nutrient) and found the variation to be high within species. He took 10 samples for each species and each plot (one plot on each island) and was therefore able to have a bigger sample size than in this experiment. Nonetheless, for one of the species included in the present study, *A. graveolens*, specific leaf area was higher for seedlings of planted under old than young forests. Specific leaf area is one of the competitive response traits and good predictors of changes in competition for resources, such as the amount of light (Violle *et al.*, 2009). The old forest in this study had a dense canopy cover compared with the young forest, thus the light condition in the understory were different. The higher specific area of *A. graveolens* planted under old forest could be a response to optimize light capture and subsequently survive better in the understory. This is evidenced from better survival rate of this species in the understory of old forest than other species (Table 2).

The results would have looked different if the sample size for each plot (sub-plot) was bigger. This can be witnessed from the tendencies in intraspecific variation observed for some of the traits. For example, *H. courbaril* seedlings, leaf thickness tended to be higher in young than old forest whereas specific leaf area and stem specific density tended to be higher in old than young forests. For seedlings of *T. decandrum*, stem dry matter content tended to be higher in young than old forest.

5.2. Do inter-specific traits of planted seedlings vary between old and young forests and trenching treatment?

Our result show that between species Lth, LDMC and SLA vary between old and young forests and SDMC was the only trait that didn't differ between species. The reason why SDMC did not differ between species could be due to a too small section of the stem measured or that it is a weak trait to predict variation between species. Among the four species in the study Lth, LDMC and SLA vary between old and young forests. SLA is higher in old forest, LDMC is higher in young forest and Lth is mostly higher in young forest than in old. The reason for these significant differences is most likely due to light availability and

different characteristics between species. SLA and LDMC is within species negatively correlated as shown in other studies (Pérez-Harguindeguy *et al.*, 2013; Cornelissen *et al.*, 2003). This suggests that increased specific leaf area allows the species to absorb light flakes that reach the understory efficiently, and hence have a better survival and growth. Density of leaves and stems were differing between species. For SSD, *D. retusa* differed from all the other species with its lower values and for LD *D. retusa* and *T. decandrum* differed with low respectively high values. This difference in density between species is probably due to different characteristics and plot heterogeneity For instance, stems of *D. retusa* quite small in many cases but sometimes bigger in some plots. *D. retusa* had quite thin leaves and the opposite was found for *T. decandrum* most of the times.

5.3. Do survival and growth of planted seedling vary in response to different environmental conditions (forest type and trenching treatments)?

For *D. retusa* the survival and growth did not vary between different environmental conditions. The rest of the species showed some variation in survival and growth with forest types, and for *H. courbaril* even variation between CAIH and trenching treatment. *A. graveolens* and *H. courbaril* showed variation in survival between the two different forest types. *A. graveolens* and *T. decandrum* showed differences between height after two consecutive years post-planting and different forest types, so did root collar diameter after one year post-planting for *A. graveolens* and root collar diameter after two year post-planting for *T. decandrum*. It was also shown that seedlings planted in the young forest had better survival and growth than the old among the species. This could be due to more light availability in the young forest than old forest. For *D. retusa* there was less variation possibly due to better adaptation. *H. courbaril* have a better height development where trenching is required which might be a case of less root competition from outside in this treatment which gives a better chance to reach nutrient and water for the seedlings within the trench.

The results showed that survival and growth vary among species and between treatments. When looking between species, it was found that survival and growth varied significantly in all cases. One major reason might be due to different characteristics between species, which lead to different types of adaptation to the environment they live in, for example some species grow faster in height, others survives better. Some species are drought tolerant (evergreen species) and some are drought avoiders (deciduous species). It is also shown that growth and survival is dependent on forest type for most of the cases, and that survival and growth was higher in the younger forest than in the old. This might be due to the light availability. The light penetrates more through the open canopy cover in the younger forest than the dense canopy of old forest. It was obvious in the field that the seedlings grew and survived better in the younger forest, the reasons could be other than light availability as well.

Height after consecutive years and diameter after one year post-planting are also dependent on the interaction between species and forest type. This shows the importance of both species

characteristics and life strategies and which condition they live in. In field all species survived and grew better in the young forest, most likely due to better light conditions and higher water- and nutrient availability. Diameter after one year post-planting was influenced by trenching treatment as well; the increased diameter growth in trenched sub-plots is most likely due to less root competition which leads to more resources (water and nutrient) available for the species inside the trench. It can also be related with the litter cover that reduces evaporation from the soil surface.

5.4. Do traits predict survival and growth?

Both intra- and inter-specific traits showed good correlations with survival and growth. Paine et al. (2015) found that functional traits are globally weak predictors of juvenile tree growth but they can be good to predict for local conditions, which supports our results to some extent. There are other studies that support this result (Martinez-Garza *et al.*, 2013; Poorter & Bongers, 2006). Many plant and tree species occupy a wide range of environmental conditions, for example spruce and pine in Sweden. This could lead to high intra-specific trait variability. High intra-specific trait variability was found for three dominant species *B. pubescens*, *V. myrtillus* and *V. vitis-idaea*, in a study on 30 islands in lakes Hornavan and Uddjaure, in Sweden (Kumordzi, 2015). Therefore I believe it is important not to look only on inter-specific trait variability (mean values of different species), since this may give an incomplete picture of how plant traits influence properties at community levels.

The negative correlation between SLA and several growth parameters shown in the results is not surprising, SLA was higher in old forest than in young forest were the growth increment was higher. Martinez-Garza (2013) studied 12 plant functional traits on 24 species (both pioneer and non-pioneer species) to see if they were good predictors of survival and/ or growth, and found that nine out of twelve traits were good predictors across all species and for pioneer species or non-pioneer species separately. It was shown that multivariate plant traits explained species growth rates and survival better than individual traits for non-pioneer species. It was also shown that LDMC was positively correlated with growth and survival when looking at non-pioneer species, and survival was negatively correlated with leaf area but for pioneer species growth is positively correlated with leaf area (Martinez-Garza *et al.*, 2013). The negative correlation between LDMC and SLA is also found in our study which is not surprising. One way to get better predictions of survival and growth could be to use a multivariable analysis were you connect different traits due to their strong relations. For example it was shown that SLA, LDMC and Lth have a strong relationship and that leaf density is equal to LDMC (Pérez-Harguindeguy *et al.*, 2013).

5.5. Limitations of the study

The small sample size (n = 4 seedlings per species and treatment) used in the original setting of the experiment is one of the limitations of the study. Particularly, the number of seedlings

used for trait measurements (n = 2) is a weakness to get conclusive intra-specific trait variability although this was feasible given the limited time, cost and low survival rate of planted seedlings. The unusually dry condition during the study period due to the El-Nino effect further exacerbated the mortality rate of seedlings; particularly for the two species, *C. vitifolium* (PoroPoro) and *S. glauca* (Aceituno), that were not included in the data analysis. Never the less, the study provides insights into the importance of both inter- and intra-specific trait variability to understand species response to environmental variability. It is also observed that different traits especially leaf traits (SLA and Lth) show high correlation with survival and growth. It is, therefore, important not to exclude within species variation that many studies do.

6. Conclusion and recommendations

Tropical dry forests are one of the most threatened ecosystem on earth and many restoration projects have failed due to limited understanding of the natural processes and a new approach is needed. In my thesis, plant functional traits were analyzed to see if they are good predictors of survival and growth of seedlings for restoration planting. The results showed that some traits especially leaf traits were good predictors of survival and growth within and between species. This is also shown by other studies like (Kumordzi, 2015); (Martinez-Garza *et al.*, 2013; Poorter & Bongers, 2006), which confirms the importance of trait-based approach to species selection for restoration project. My findings highlight the variations in functional traits, survival and growth within and between species in different environmental conditions. *A. graveolens* showing the best growth and survival rate and are also the only species showing variation between traits (SLA) and different environmental conditions when looking on within species variation. Within species *A. graveolens* also showing the highest correlation between leaf traits and CAIH and CAID. *A. graveolens* seems to be a good species to use in restoration projects in SDTF conditions similar to this experiment. There are not many studies made on functional traits in seasonally dry tropical forests, therefore new studies is needed in this topic. As sample sizes were small in the present study, I recommend further research using larger sample sizes for each treatment (sub-plot) and more species to be able to identify the best predictive traits. It could also be interesting to make a multivariable analysis in future research, to connect different traits due to their strong relations, for example the relation between SLA, LDMC and Lth.

References

ACG, published 2012-07-27, last update 2014-03-10, accessed 2015-06-22, ACG Programa de Restauracion y Silvicultura. http://www.acguanacaste.ac.cr/biodesarrollo/programa-de-restauracion-y-silvicultura. www.acguanacaste.ac.cr;

http://www.acguanacaste.ac.cr/acg/historia, accessed: 2015-06-27

Alder, P.S.-G., R.; Compagnonia, A.; Hsu, J.; Ray-Mukherjee, J.; Mbeau-Ache, C.; and Franco, M. (2014). Correction for Adler et al., Functional traits explain variation in plant life history strategies. *Proceedings of the National Academy of Sciences*, 111(27), pp. 10019-10019.

Asanok, L., Marod, D., Duengkae, P., Pranmongkol, U., Kurokawa, H., Aiba, M., Katabuchi, M. & Nakashizuka, T. (2013). Relationships between functional traits and the ability of forest tree species to reestablish in secondary forest and enrichment plantations in the uplands of northern Thailand. *Forest Ecology and Management*, 296, pp. 9-23.

Arboles de Centroamerica (AdC), 2012-11-17. Mesoamerican Tree Species: a source book for farm planting and ecological restoration. AdC. Accessed: 2015-10-27. www.arbolesdecentroamerica.info/index.php/en/species

Birouste, M., Zamora-Ledezma, E., Bossard, C., Pérez-Ramos, I.M. & Roumet, C. (2013). Measurement of fine root tissue density: a comparison of three methods reveals the potential of root dry matter content. *Plant and Soil*, 374(1-2), pp. 299-313.

Calvo-Alvarado, J., McLennan, B., Sánchez-Azofeifa, A. & Garvin, T. (2009). Deforestation and forest restoration in Guanacaste, Costa Rica: Putting conservation policies in context. *Forest Ecology and Management*, 258(6), pp. 931-940.

Castellanos Castro, C. & Newton, A.C. (2015). Leaf and Stem Trait Variation and Plant Functional Types in 113 Woody Species of a Seasonally Dry Tropical Forest. *Colombia Forestal*, 18(1).

Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G. & Zanne, A.E. (2009). Towards a worldwide wood economics spectrum. *Ecol Lett*, 12(4), pp. 351-66.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., ter Steege, H., Morgan, H.D., van der Heijden, M.G.A., Pausas, J.G. & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, 51(4), pp. 335-380.

Craine, J.M., Dybzinski, R. & Robinson, D. (2013). Mechanisms of plant competition for nutrients, water and light. *Functional Ecology*, 27(4), pp. 833-840.

Croat, T. 1978. The Flora of Barro Colorado Island. Stanford University Press, ISBN 0-8047-0950-5. Flora of Barro Colorado Island, Smithsonian Tropical Research Institute. Accessed: 2015-10-27. http://biogeodb.stri.si.edu/bioinformatics/croat/home

Garnier, E. & Navas, M.-L. (2011). A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. *Agronomy for Sustainable Development,* 32(2), pp. 365-399.

Global forest watch, source: Hansen/UMD/Google/USGS/NASA. Accessed: 2015-06-22. http://www.globalforestwatch.org/country/CRI

Griscom, H.P. & Ashton, M.S. (2011). Restoration of dry tropical forests in Central America: A review of pattern and process. *Forest Ecology and Management,* 261(10), pp. 1564-1579.

Hulshof, C.M. & Swenson, N.G. (2010). Variation in leaf functional trait values within and across individuals and species: an example from a Costa Rican dry forest. *Functional Ecology,* 24(1), pp. 217-223.

Krober, W., Heklau, H. & Bruelheide, H. (2015). Leaf morphology of 40 evergreen and deciduous broadleaved subtropical tree species and relationships to functional ecophysiological traits. *Plant Biol (Stuttg),* 17(2), pp. 373-83.

Kuparinen, A. (2006). Mechanistic models for wind dispersal. *Trends in Plant Science,* 11(6), pp. 296-301.

Laughlin, D.C., Leppert, J.J., Moore, M.M. & Sieg, C.H. (2010). A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. *Functional Ecology,* 24(3), pp. 493-501.

Lopez-Iglesias, B., Villar, R. & Poorter, L. (2014). Functional traits predict drought performance and distribution of Mediterranean woody species. *Acta Oecologica-International Journal of Ecology,* 56, pp. 10-18.

Markesteijn, L. & Poorter, L. (2009). Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *Journal of Ecology,* 97(2), pp. 311-325.

Martinez-Garza, C., Bongers, F. & Poorter, L. (2013). Are functional traits good predictors of species performance in restoration plantings in tropical abandoned pastures? *Forest Ecology and Management,* 303, pp. 35-45.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S. & Cornelissen, J.H.C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany,* 61(3), p. 167.

Poorter, L. & Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology,* 87(7), pp. 1733-1743.

Poorter, L. & Markesteijn, L. (2008). Seedling traits determine drought tolerance of tropical tree species. *Biotropica,* 40(3), pp. 321-331.

Poorter, L., Wright, S.J., Paz, H., Ackerly, D.D., Condit, R., Ibarra-Manriques, G., Harms, K.E., Licona, J.C., Martinez-Ramos, M., Mazer, S.J., Muller-Landau, H.C., Pena-Claros, M., Webb, C.O. & Wright, I.J. (2008). Are functional traits good predictors

of demographic rates? Evidence from five Neotropical forests. *Ecology*, 89(7), pp. 1908-1920.

Powers, J.S. & Tiffin, P. (2010). Plant functional type classifications in tropical dry forests in Costa Rica: leaf habit versus taxonomic approaches. *Functional Ecology*, 24(4), pp. 927-936.

Reich, P.B., Wright, I.J., Cavender-Bares, J., Craine, J.M., Oleksyn, J., Westoby, M. & Walters, M.B. (2003). The evolution of plant functional variation: Traits, spectra, and strategies. *International Journal of Plant Sciences*, 164(3), pp. S143-S164.

Ruger, N., Wirth, C., Wright, S.J. & Condit, R. (2012). Functional traits explain light and size response of growth rates in tropical tree species. *Ecology*, 93(12), pp. 2626-2636.

SER 2004. Society for Ecological Restoration International Science & Policy Working Group. 2004. The International Primer on Ecological Restoration. www.ser.org & Tucson: Society for Ecological Restoration International. Accessed: 2016-01-05. http://www.ser.org/

Schönbeck, L., Lohbeck, M., Bongers, F., Ramos, M. & Sterck, F. (2015). How do Light and Water Acquisition Strategies Affect Species Selection during Secondary Succession in Moist Tropical Forests? *Forests*, 6(6), pp. 2047-2065.

Souza, B.C.d., Oliveira, R.S., Araújo, F.S.d., Lima, A.L.A.d. & Rodal, M.J.N. (2015). Divergências funcionais e estratégias de resistência à seca entre espécies decíduas e sempre verdes tropicais. *Rodriguésia*, 66(1), pp. 21-32.

Stanturf, J.A., Schoenholtz, S.H., Schweitzer, C.J. & Shepard, J.P. (2001). Achieving restoration success: Myths in bottomland hardwood forests. *Restoration Ecology*, 9(2), pp. 189-200.

The global partnership on forest and landscape restoration. 2013. http://www.forestlandscaperestoration.org/ accessed: 2015-09-16.

Tunjai, P. & Elliott, S. (2012). Effects of seed traits on the success of direct seeding for restoring southern Thailand's lowland evergreen forest ecosystem. *New Forests*, 43(3), pp. 319-333.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012). The return of the variance: intraspecific variability in community ecology. *Trends Ecol Evol*, 27(4), pp. 244-52.

Violle, C., Garnier, E., Lecoeur, J., Roumet, C., Podeur, C., Blanchard, A. & Navas, M.L. (2009). Competition, traits and resource depletion in plant communities. *Oecologia*, 160(4), pp. 747-755.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I. & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, 116(5), pp. 882-892.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G.,

Veneklaas, E.J. & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature,* 428(6985), pp. 821-827.

Wright, S. J., Bunker, D., Dalling, J., Davies, S., Díaz, S. et al. 2006. Towards a functional trait based research program within the Center for Tropical Forest Science. Smithsonian Tropical Research Institute, Center for Tropical Forest Science, CTFS. Accessed: 2015-10-26 www.ctfs.si.edu/data/documents/Traits_report_20060817.pdf